Chapter 5

Algorithm to Simulate a Chemically Induced DNA Logic Gate and Boolean Circuit

5.1 An Overview

The structural switching of single stranded DNA to several secondary structures opens a new gate simulation strategy [74–83] with few added advantages such as faster response time, simple design, reusability and easy handling procedures. Recently Henry Albert Day et al. [84] have revealed in their research paper about the three structural switching property of C- rich DNA strand. Initially the linear single stranded DNA folds to i-motif structure either at acidic pH or in neutral pH (in presence of certain cations or ligands). The i-motif structure is further switched to hairpin structure in presence of copper(II) cations. The copper ion induced hairpin structure could be reversed to previous i-motif conformation by chelation with Ethylenediaminetetraacetic acid (EDTA). In their experimental paper they

revealed that the same oligonucleotide sequence has the potential to attain three conformation i.e. linear, i-motif and hairpin without changing the pH. The actual binding mechanism of Cu^{2+} to DNA is not known but it is speculated that copper(II) cation binds to sugar phosphate backbone. In this chapter the idea is extended to demonstrate simulation model for DNA based AND and OR logic gate.

Apart from the most common B form, DNA can adopt other conformations such as A form, D form, Z form etc. DNA has the potential to fold into non canonical structures such as triplex (H-DNA), tetraplex (G-quadruplex) and i-motif which are extensively studied by researchers in the field of nanotechnology. Specific structure of i-motif conformation is obtained by spatial arrangement of intercalated base pairs holded by three hydrogen bonds between one neutral cytosine(C) and one protonated C+ at N3 in a C rich nucleic acid chain. The C.C+ base pair interaction is stronger than G.C base pair i.e. Base-Pairing Energy (BPE) of C.C+ is 169.7 kJ /mol whereas BPE of G.C and neutral C.C is 96.6 kJ/mol and 68.0 kJ/mol respectively [94]. i-motif structure can be either intramolecular or intermolecular. In intramolecular, i-motif is formed from base pairs involving C tracts present in one strand whereas in intermolecular i-motif is formed when C.C+ base pairs are established between two or four independent strands. **Figure 5.1** shows the C.C+ base pair in a i-motif structure.

The position of hydrogen bonded to the two N3s in each C.C+ base pair is not equidistant rather it is positioned to the farthest hydrogen atom of the next C.C+ pair [95]. In i-motif the interaction between consecutive base pairs are provided by stacking of the exocyclic carbonyl and amino groups but the aromatic heterocycles of the bases does not participate in the staking. pH of the solution plays an important role during i-motif formation by protonating one of the C bases in the C.C+ bond. It has been found that C bases are partially protonated at the range of pH from $\sim$4 to $\sim$7 and 25^{o}C hence detected a conformation change to i-motif.

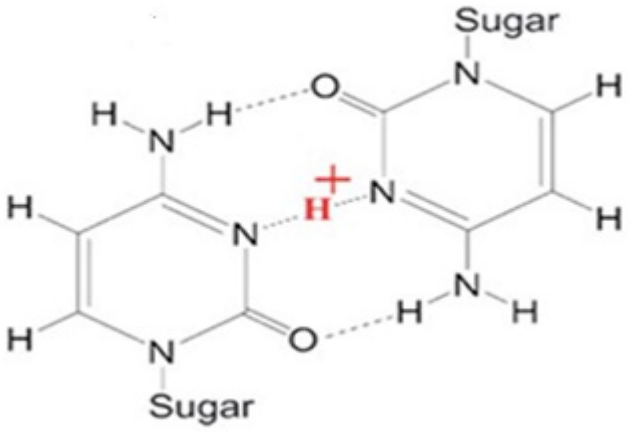

Figure 5.1: The C.C+ base pairing in a i-motif structure.

As the pH value gets higher the C bases deprotonates and as a result the structure unfolds to a single-stranded form whereas on the other hand at lower pH i.e. at pH below 3 all the C bases are protonated and hence could not form C.C+ base pair in absence of C bases needed for the bond [96]. Hence at pH lower than 4 and higher than 7 no stable i-motif structure exists. Experimental evidence is there in justification of the concept that Cu^{2+} contributes in stabilizing the sugar-phosphate backbone in i-motif to induce hairpin structure [84]. The C-rich DNA strand can be reused up-to multiple cycle of operation by regulating EDTA and pH in the solution. EDTA chelate the Cu^{2+} ions due to which the hairpin structure is reverse to previous i-motif form and again the i-motif conformation can be further reversed to linear form by regulating the pH of the solution.

In the following sections algorithms for simulation of AND, OR and Boolean circuit is described.

5.2 AND gate Simulation

AND gate generates an output value '1' only if all of its input values are "true" otherwise generates '0' as output. The functionality of AND gate can be considered as serial connection. In this work a theoretical realization of AND gate is demonstrated at molecular level by regulating H^+ and Cu^{2+} ($CuCl_2$) content. The entire process can be represented in the form of an algorithm (Algorithm 5) as shown below:

Algorithm 5 : Algorithm for AND gate

AND_operator(gate_strand, T_1)
{
$T_1 \leftarrow T_1 \cup$ gate_strand;
if ($X_1 == 0$) **then**
{
No titration of H^+ to T_1; // *No change in the structure of gate_strand*
if ($X_2 == 0$ || $X_2 == 1$) **then**
{
No Cu^{2+} is added; // *No change in the structure of gate_strand*
Output = 0;
}
end if
else
if ($X_1 == 1$) **then**
{
No titration of H^+ to T_1; // *No change in the structure of gate_strand*
if ($X_2 == 1$) **then**
Add Cu^{2+} to T_1; // *i-motif structure change to hairpin structure*
Output = 1;
else
No Cu^{2+} is added; // *No Change in the i-motif structure*
Output = 0;
end if
}
end if
}
end if

In the above algorithm the *gate_strand* is the C-rich DNA strand 5'-TAA-CCC-TAA-CCC-TAA-CCC-TAA-CCC-3'which is used for simulation process. Depending on the inputs in the form of either H^+ or Cu^{2+} or both several conformational changes are observed and accordingly output is read. T_1 is the test tube where the reactions are carried out. The output is read as '1' when the structure of *gate_strand* attain the hairpin shape which occurs only when both the inputs are present and in absence of any of the input the structure of gate_strand remains in either linear form or in i-motif form which is read as output 0. X_1 and X_2 are the two inputs of DNA-AND gate. The first input variable is associated to H^+ ion and the second input variable is associated with Cu^{2+} ion. **Table 5.1** shows truth table of AND gate with H^+ and Cu^{2+} as its inputs. The AND gate (shown in **Figure 5.2**) simulation is illustrated in **Figure 5.3** to **Figure 5.6**. During this simulation process the order of providing the input is very important e.g. H^+ followed by Cu^{2+} is not same as Cu^{2+} followed by H^+.

Table 5.1: Two input AND gate.

X_1 (H^+)	0	0	1	1
X_2 (Cu^{2+})	0	1	0	1
Output	0	0	0	1

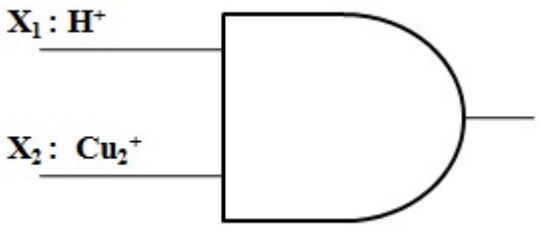

Figure 5.2: AND gate with two inputs.

The theoretical simulation results of DNA-AND gate is illustrated below for the four cases i.e. Case a: (0, 0), Case b: (0, 1), Case c: (1, 0) and Case d:(1, 1).

Case a: (0, 0) Simulation

When both inputs are absent i.e. $X_1 = 0$ (H^+ is absent) and $X_2 = 0$ (Cu^{2+} is absent), no conformational change in observed in the C rich DNA sequence 5'-TAACCCTAACCCTAACCCTCACCCTAA-3'as shown in **Figure 5.3** hence the output is read as 0 which is in agreement with digital AND gate.

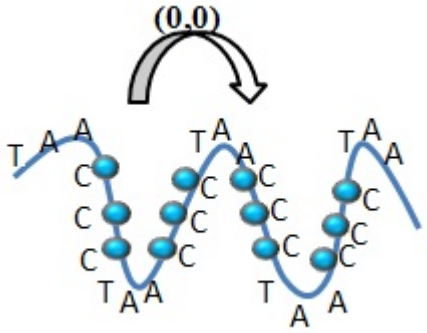

Figure 5.3: DNA-AND gate simulation for input Case a: (0, 0).

Similarly, theoretical validation is shown for all other digital input cases i.e. Case b: (0, 1), Case c: (1, 0) and Case d: (1, 1). **Table 5.2** shows the result of molecular simulation of AND gate.

Case b: (0, 1) Simulation

Inputs (0, 1) i.e. when the input X_1 is present and X_2 is absent. As ordering of inputs plays an important role therefore this case is not similar to **Case c:** (1, 0). In absence of hydrogen ion there is no switching of linear to i-motif structure hence there is no hairpin structure observed as shown in **Figure 5.4**.

Case c: (1, 0) Simulation

In **Case c**, the first input X_1 i.e. H^+ is present and the second input $X_2 = Cu^{2+}$ is absent. Initially when hydrogen ions are provided to the test tube T_1 containing gate strands, conformational change of linear to i-motif is detected as shown in **Figure 5.5**. But as the second input i.e. Cu^{2+} is absent no further change of structure is detected.

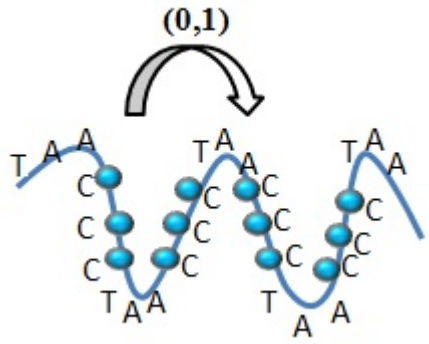

Figure 5.4: DNA-AND gate Simulation for input Case b: (0, 1).

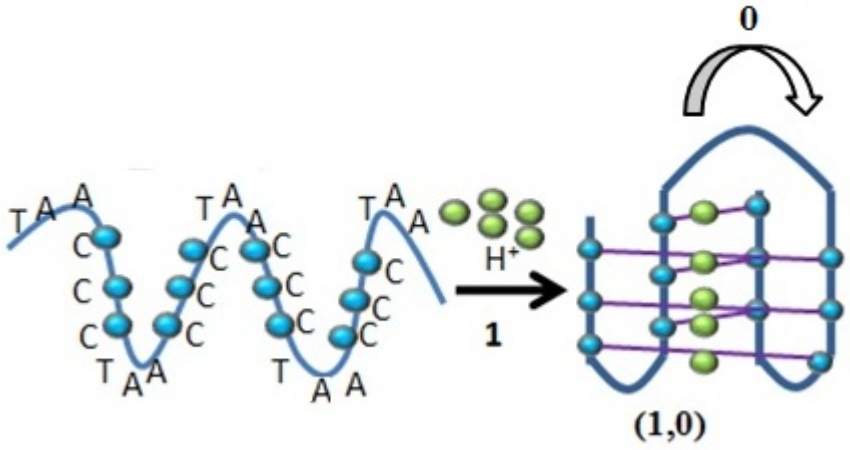

Figure 5.5: DNA-AND gate Simulation for input Case c: (1, 0).

Case d: (1, 1) Simulation

In **Case d** the input (1, 1) is provided in the form of H^+ and Cu^{2+} ions. Initially in presence of hydrogen ion the linear strand change to i-motif and later on changes to hairpin structure in presence of Cu^2+ as shown in **Figure 5.6**.

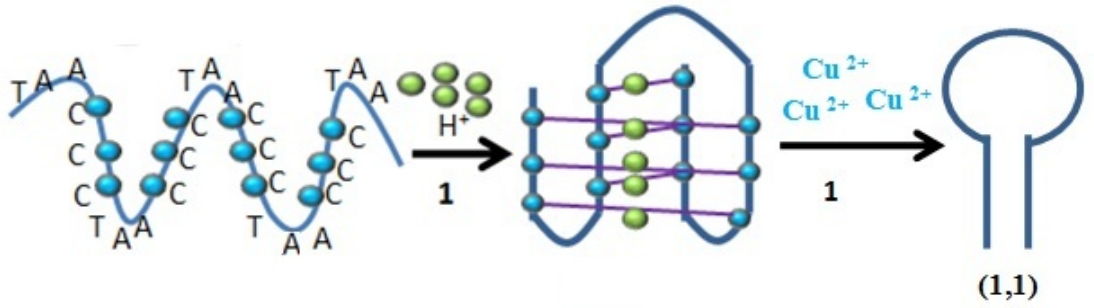

Figure 5.6: DNA-AND gate Simulation for input Case d: (1, 1).

Table 5.2: Molecular simulation of AND gate.

H^+	Cu^{2+}	Simulation Output	Digital Output
0	0	Linear	0
0	1	Linear	0
1	0	i-motif	0
1	1	Hairpin	1

5.3 OR gate Simulation

OR gate (shown in **Figure 5.7**) evaluates to '1' if any of its inputs are "true" otherwise gives '0' as output. Unlike AND gate where H^+ and Cu^{2+} acts as inputs, OR gate simulation is realized regulating only H^+ in the test tube. **Table 5.3** shows truth table of OR gate with H^+ as its two inputs.The entire process can be represented in the form of an algorithm (illustrated in Algorithm 6):

gate_strand is a C-rich strand having potential to attain i-motif structure (5'-TAA-CCC-TAA-CCC-TAA-CCC-TAA-CCC-3'). When H^+ is added to a test tube containing population of DNA sequence (*gate_strand*), the pH of the solu-

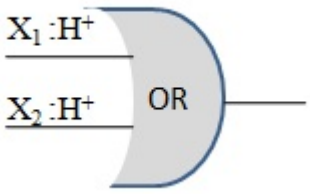

Figure 5.7: OR gate with two inputs.

Algorithm 6 : Algorithm for OR gate

OR_operator(gate_strand, T_1)
{
$T_1 \leftarrow T_1 \cup$ gate_strand;
if ($X_1 == 1$ || $X_2 == 1$ || $X_1 == 1$ && $X_2 == 1$) **then**
Titrate H^+ to T_1; // *gate strand change to i-motif structure*
Output = 1;
else
No H^+ is titrated to T_1; // *No change to gate strand*
Output = 0;
end if
}

tion changes to acidic and hence C bases starts protonating resulting in i-motif structure. The output is read as '1' when the structure of *gate_strand* attains i-motif structure. **Table 5.4** shows the result of molecular simulation of OR gate.

Table 5.3: Two input OR gate.

X_1 (H^+)	0	0	1	1
X_2 (H^+)	0	1	0	1
Output	0	1	1	1

Case a: (0, 0) Simulation

For OR gate when both inputs are absent i.e. $X_1 = 0$ and $X_2 = 0$ no H^+ is added as a result no conformational change in observed in the C rich DNA gate_strand

sequence as shown in **Figure 5.8** hence the output is read as '0'.

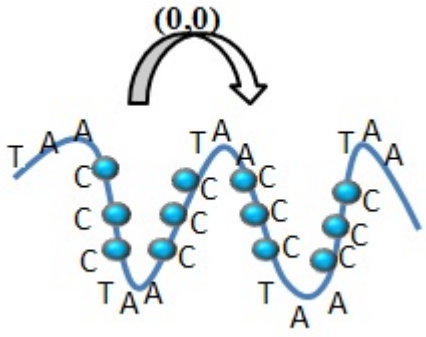

Figure 5.8: DNA-OR gate Simulation for input Case a: (0,0).

Case b: (0, 1) Simulation

When the input are $X_1 = 0$ and $X_2 = 1$, conformation change is observed with detection of transition of linear strand to i-motif structure as shown in **Figure 5.9**. The existence of i-motif evaluates the output as '1'.

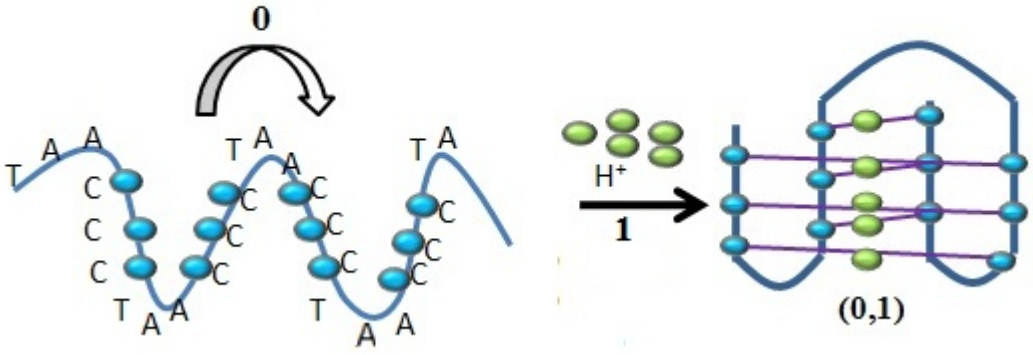

Figure 5.9: DNA-OR gate Simulation for input Case b: (0,1).

Case c: (1, 0) Simulation

In **Case c**, the first input X_1 is present and the second input X_2 is absent. When hydrogen ions are provided to the test tube T_1 containing gate strands conformational change of linear to i-motif is detected which read as output '1' as shown in **Figure 5.10**.

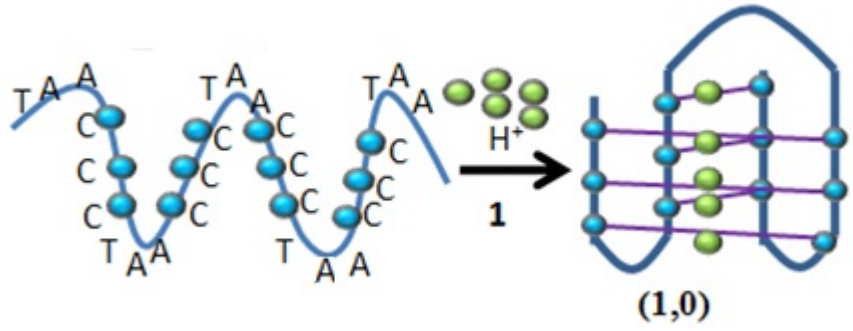

Figure 5.10: DNA-OR gate Simulation for input Case c: (1,0).

Case d: (1, 1) Simulation

In this case both of the inputs are present. In presence of hydrogen ion the linear strand changes to i-motif which read as output '1' as shown in **Figure 5.11**.

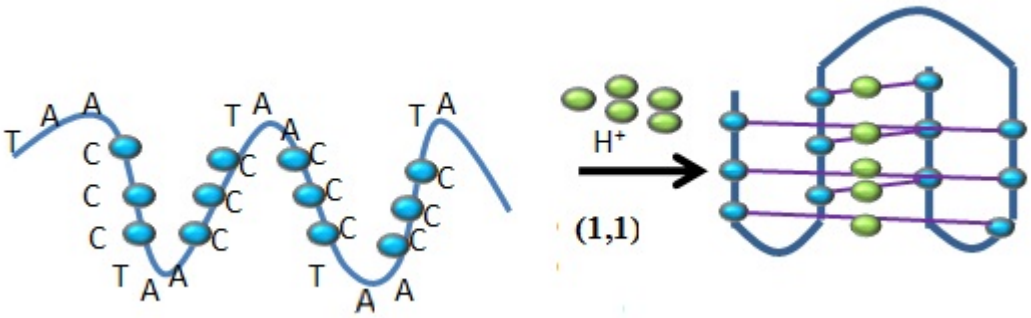

Figure 5.11: DNA-OR gate Simulation for input Case d: (1,1).

Table 5.4: Molecular simulation of OR gate.

H^+	H^+	Simulation Output	Digital Output
0	0	Linear	0
0	1	i-motif	1
1	0	i-motif	1
1	1	i-motif	1

5.4 Boolean Circuit Evaluation

A Boolean circuit can be visualized as directed acyclic graph with all the gates as nodes connected to each other in such a way that the output of one level serves as input to the higher level. **Figure 5.12** illustrates a three leveled Boolean circuit with Level 0, Level 1 and Level 2 are inputs, intermediate gates (AND and OR) and output gate (AND) respectively. The algorithm to emulate a Boolean circuit is shown in Algorithm 7, where *level_max* represents maximum level in the circuit and *gate_max* represents maximum number of gates in each level.

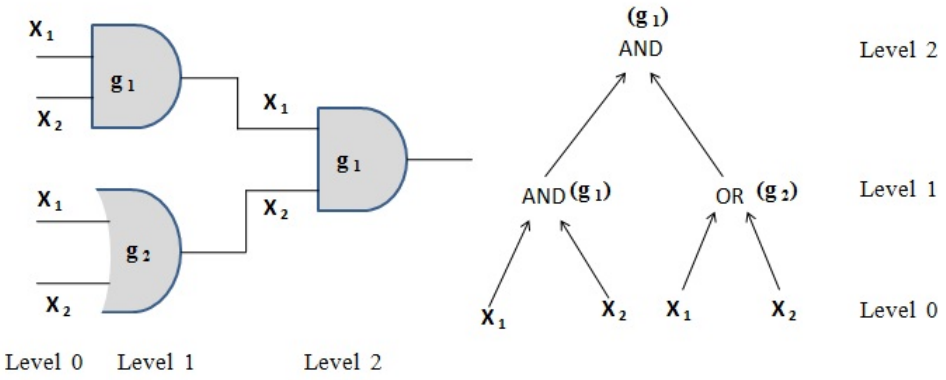

Figure 5.12: Instance of a AND-OR Boolean circuit.

Algorithm 7 : Algorithm for AND-OR Boolean Circuit

```
Boolean_operator(gate_strand, T1)
for (j = 1 to j = level_max) do
  for (k = 1 to k ≤ gate_max) do
    if (gk = = AND) then
      AND_operator(gate_strand, Tk)
    end if
    if (gk = = OR) then
      OR_operator(gate_strand, Tk)
    end if
  end for
end for
```

The simulation of an n-leveled Boolean circuit with k number of logic gates

required k numbers of test tubes. Each of the logic function is carried out in different test tubes and the output generated in each test tube is fetched as input to the gates in next level with human intervention. To validate the evaluation of Boolean circuit by using the proposed algorithm, an instance of a circuit with inputs (1,1) to g_1 and (0,0) to g_2 is evaluated. As all the logic operation in each gate is executed in different test tubes, a maximum of three test tubes are required to evaluate this Boolean circuit.

Level 1 Simulation

The two gates of level 1 i.e. AND (g_1) and OR (g_2) are encoded in the form of C-rich DNA strand (*gate_strand*; 5'-TAACCCTAACCCTAACCCTCACCCTAA-3'). Inputs are provided to the test tubes in the form of H^+ and Cu^{2+} ions.

The g_1 is AND gate with inputs $X_1 = 1$, $X_2 = 1$, therefore the input for the gate operation are provided in the form of presence of H^+ ion and Cu^{2+} ions. The simulation is similar to **Case d**: (1, 1) of AND gate and hence the output generated is '1'. Similarly, for OR gate (g_2) with inputs $X_1 = 0$, $X_2 = 0$, no conformational change in the *gate_strand* is detected and hence the output generated is '0'. The output generated at test tube T_1 is read as '1' since the final conformation attained by the *gate_strand* is hairpin whereas the simulation for g_2 at test tube T_2 witness no conformational change in *gate_strand* which evaluates output as '0'. The outputs of gates at Level 1 acts as input to the gate at Level 2 (shown in **Figure 5.13**).

Level 2 Simulation

Level 2 consists of AND gate (g_3). Inputs to g_3 are output of g_1 and output of g_2 i.e. (1,0). The theoretical simulation demonstrated in **Figure 5.13**.

The final output agrees with the digital circuit evaluation and therefore it can be stated that the proposed algorithm is applicable to simulate any Boolean circuit at the molecular level. As the time incurred for each cycle of switching is less than 100 seconds, it can be concluded that the proposed logic gate has fast response

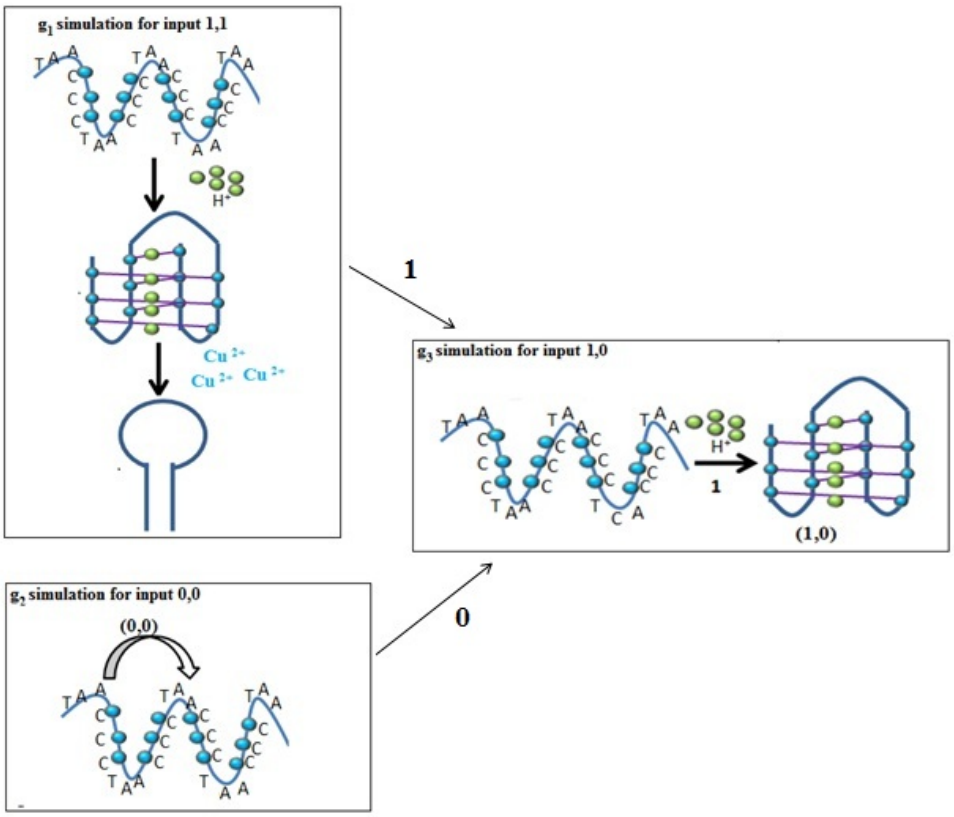

Figure 5.13: AND-OR Boolean circuit simulation at molecular level.

time. Similarly the model is reusable as several cycles of switching are possible for same strand of DNA as the linear strand can be restored every time by controlling the amount and concentration of pH, Cu^{2+} or EDTA in the solution.

The model exploits the potential of three different structural outputs (linear, i-motif, hairpin) on the basis of pH condition and cationic concentration in the solution which provide a new insight to design DNA logic gate and Boolean circuits.

5.5 Summary

A new gate design strategy is proposed to simulate AND and OR gate individually as well as to evaluate any AND-OR Boolean circuit by controlling H^+ and / or

Cu^{2+}. The conformational change of C-rich DNA strand to i-motif structure and hairpin structure at acidic pH and physiological temperature is utilized throughout the operations. The advantage of such chemically induced gates are faster response time, simple design, reusability and easy handling procedures.